

Scanning Electron Microscopy in the Evaluation of Defects to the Ossicular Chain in the Course of Chronic Otitis Media

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Abstract

Objective: Chronic otitis media is a heterogeneous disorder. Chronic suppurative otitis media with cholesteatoma and, to a lesser extent, chronic otitis media with granulation lead to the destruction of bone structures within the middle ear. Bone loss may appear in the prominence of the horizontal semicircular canals and the bony canal of the facial nerve. The inflammatory process may spread to the bony labyrinth of the sigmoidal sinuses and the cranial cavities. **Materials and Methods:** The analysis comprised the examination of fragments of auditory ossicles removed during surgery in 21 patients with various types of chronic inflammation of the middle ear. The purpose of this study was to evaluate the usefulness of scanning electron microscopy in evaluating the erosion of middle ear ossicles in different types of chronic otitis media. Images captured at various magnifications were used for the best possible illustration of the observed lesions in bone tissue. **Results:** The observed lesions and the degree of bone surface damage were dependent on the type of chronic inflammation of the middle ear. The largest destructive changes in the ossicular chain were observed in chronic otitis media with cholesteatoma; smaller changes were observed in chronic otitis media with granulation. **Conclusion:** In the case of damage to the ossicles inflicted by inflammation, the use of a modeled bone block made of a temporal bone taken from outside the tympanic cavity or a biomaterial prosthesis is a good choice in ossiculoplasty.

Keywords

scanning electron microscopy, ossicular chain defects, chronic otitis media

Introduction

Chronic otitis media is characterized by loss of the tympanic membrane, occasional otorrhea, and various conductive hearing impairments. The pathogenesis of this disease is complex. At its base is biological worsening of the immunological resistance of the middle ear lining, past bouts of inflammation, the type and resistance of the strain of bacteria causing infection, anatomical conditions of the middle ear pneumatic spaces, and general diseases, such as allergies, immunodeficiency syndromes, and diabetes.

Chronic otitis media is a heterogeneous disorder. In the active form, depending on the observed lesions of the middle ear lining, the following varieties may be distinguished: simple chronic otitis media, chronic otitis media with granulation, chronic otitis media with cholesteatoma, and chronic otitis media in the course of specific diseases.

Chronic suppurative otitis media with cholesteatoma (otitis media purulenta chronica cholesteatomatous) and, to a lesser extent, chronic otitis media with granulation (otitis media

granulomatosa) lead to the destruction of bone structures within the middle ear.

The destructive effect of chronic suppurative otitis media with cholesteatoma in the middle ear initially consists of destruction of the ossicular chain through bone resorption. The process of bone resorption in cholesteatoma is initiated by the molecular cascade of osteoclast differentiation factors, which include osteoprotegerin (OPG), receptor activator of nuclear factor κ B (RANK), located on osteoclasts, and receptor activator for nuclear factor κ B ligand (RANKL), located on the

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surface of osteoblasts. This system is the most important cascade regulating the process of bone destruction. The long process of the incus is the quickest to erode. Bone loss may appear in the prominence of the horizontal semicircular canals and the bony canal of the facial nerve. The inflammatory process may spread to the bony labyrinth of the sigmoidal sinuses and the cranial cavities.

In chronic otitis media with granulation, the pathological granulation tissue contains inflammatory cells, such as T and B lymphocytes, plasma cells, and other cells from the monocyte-macrophage line, producing large quantities of pro-inflammatory cytokines (including interleukin 1 α [IL-1 α], IL-1 β , IL-6, prostaglandin E2, and tumor necrosis factor α [TNF- α]). Studies have shown significantly increased levels of RANKL, the main source of which is activated T cells, and reduced levels of OPG in the granulation tissue adhering to the bone. The RANKL-OPG-RANK system is crucial in the process of bone resorption in all chronic inflammatory conditions, and the cascade of other inflammatory signals activates, regulates, and supplements the mechanism of the inflammatory process.¹⁻⁵

Materials and Methods

The analysis comprised the examination of fragments of auditory ossicles removed during surgery in 21 patients with various types of chronic inflammation of the middle ear. The fragments of ossicles were not suitable for use as a reconstructive material for the middle ear conduction system or were damaged due to the very advanced inflammatory process.

At the same time, histopathological examination was performed. Based on the clinical state of the middle ear verified during surgery and the obtained histopathological results, all 21 patients participating in the study were divided into 3 groups (Table 1).

For the purpose of assessing the structural properties of the middle ear ossicles and the advancement of the inflammatory process, the surgically removed material was fixed in 4% formalin and dehydrated in an ethanol series of 35%, 50%, 75%, 96%, and 99.9% (pure alcohol). Once prepared, the samples were air-dried and mounted on slides using glue (Electrodag 915 Silver Paint; Ted Pella, Redding, California). The prepared samples were sputter-coated with a thin layer of gold using a JEOL JEE-4x device (JEOL JEE-4x; JEOL Vacuum Evaporator, Freising, Germany; Figure 1).

Subsequently, analysis of the prepared samples was performed using a JEOL JSM-35CF scanning microscope (JSM-35CF; JEOL Vacuum Evaporator). The degree of damage to the ossicle surface at different magnifications was assessed depending on the type of chronic inflammation. To conduct a comparative analysis of the surface of the examined samples, magnifications of $\times 36$, $\times 100$, $\times 200$, and $\times 2000$ were selected, and the samples in individual groups (I, II, III) were compared.

Informed consent was obtained from each patient included in the study. The local ethics committee approved the study.

Table 1. Division of Patients Into Groups Depending on the Type of Chronic Inflammation of the Middle Ear.

Type of Chronic Inflammation	Group Size, Symbol of Patients, The Analyzed element of the ossicular chain
Chronic suppurative otitis media with cholesteatoma	Group I (7 patients) P1—patient no. 1 P2—patient no. 2 P3—patient no. 3 P4—patient no. 4 P5—patient no. 5 P6—patient no. 6 P7—patient no. 7
Chronic otitis media with granulation	Group II (7 patients) P8—patient no. 8 P9—patient no. 9 P10—patient no. 10 P11—patient no. 11 P12—patient no. 12 P13—patient no. 13 P14—patient no. 14
Simple chronic otitis media	Group III (6 patients) P 15—patient no. 15 P 16—patient no. 16 P 17—patient no. 17 P 18—patient no. 18 P 19—patient no. 19 P 20—patient no. 20 P 21—patient no. 21



Figure 1. A prepared bone sample sputter-coated with a gold layer for examination by scanning electron microscopy.

Statistical Analysis

In the statistical analysis process, Statistica software was used (StatSoft Polska, Krakow, Poland).

Results

Images captured at various magnifications were obtained for the best possible illustration of the observed lesions in bone tissue. To consolidate the results, scanning electron microscopy

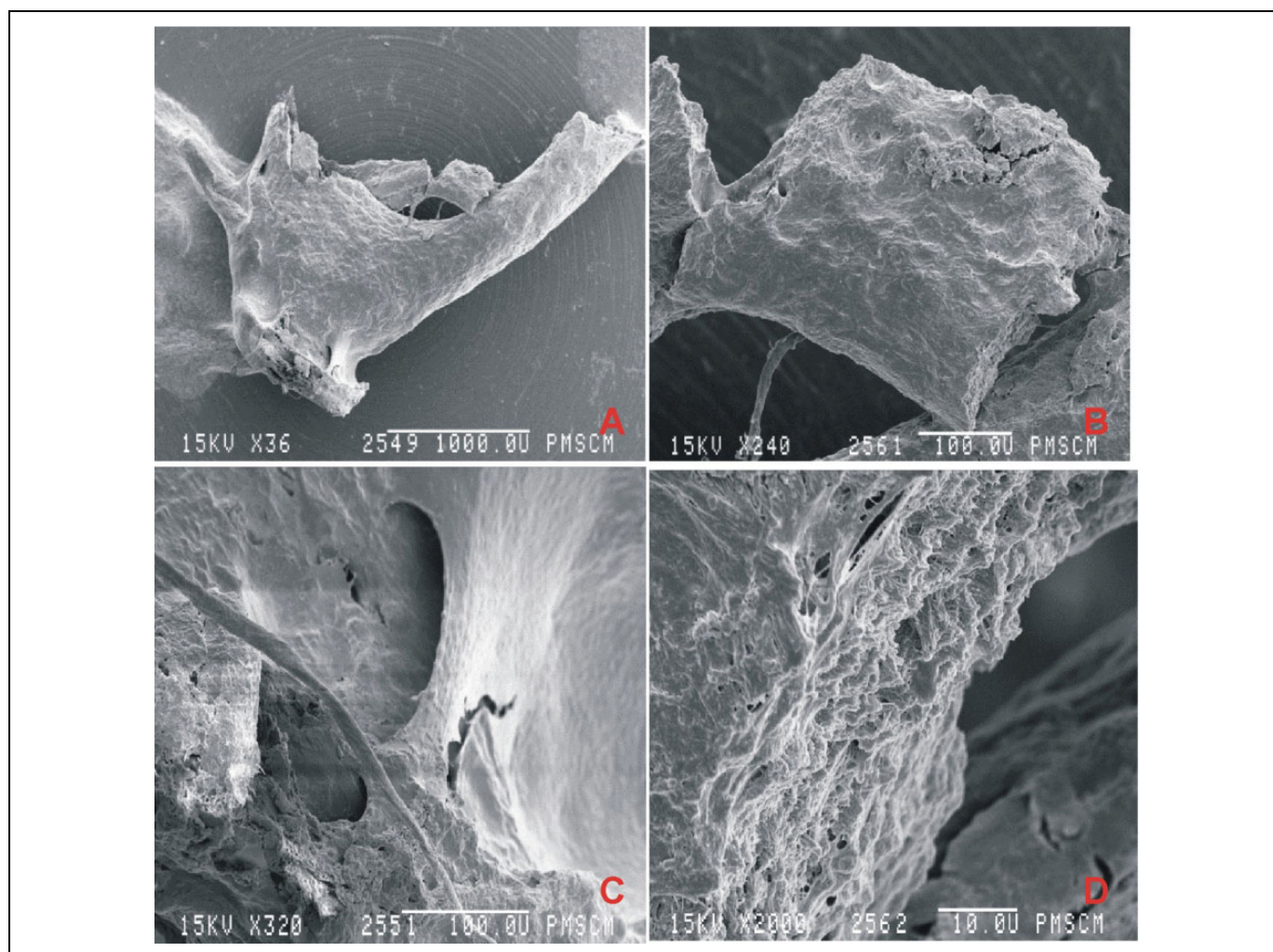


Figure 2. Chronic cholesteatoma otitis media. Removed stapes (group I, PI). Scanning electron microscopy showed a fragment of a damaged stIRRUP (A) $\times 36$, broken anterior crus (B) $\times 240$, vicinity of the stapes' head (C) $\times 320$, and end of the anterior crus (D) $\times 2000$.

(SEM) images obtained at magnifications of $\times 36$, $\times 100$, $\times 200$, and $\times 2000$ were analyzed.

Group I: Chronic suppurative otitis media with cholesteatoma (Figures 2–4)

Group II: Chronic otitis media with granulation (Figures 5–7)

Group III: Simple chronic otitis media (Figures 8–10)

The observed lesions and the degree of bone surface damage are summarized in Table 2 to compare bone damage depending on the type of chronic inflammation of the middle ear.

Discussion

The process of resorption and reconstruction in chronic inflammation of the middle ear is responsible for lesions in bone tissue of the ossicular chain. Remodeling and reconstruction are based on the cyclical processes of bone resorption and formation occurring one after the other, activated and modified by a number of inflammatory factors. The degree of bone tissue damage largely depends on the type of chronic inflammation,

its duration and the extent of inflammatory lesions, or the etiology of inflammation (bacterial, fungal). The greatest damage is observed, which is consistent with our observations, in the case of cholesteatoma of the middle ear. Research to explain the mechanisms of soft tissue and bone damage in this type of inflammation was undertaken by Virchow, who, at the end of 1864, was the first to show the destructive effect of cholesteatoma on bone. The expansive effect of cholesteatoma causing bone destruction via the mechanism of pressure and thus bone destruction was supported in 1951 by Walsh.⁶ This theory was overturned in 1987 after studies by Orisek and Chole,⁷ who showed that the pressure in the capillaries under the cholesteatoma varied from 1.3 to 11.9 mm Hg (normal: 25 mm Hg). Thomsen et al⁸ and Chole⁹ have shown that acid phosphatase is responsible for bone resorption in cholesteatoma, and lowering the pH promotes the demineralization of hydroxyapatite. Moriyama et al¹⁰ have found that cholesteatoma induces bone resorption by altering the electrical potential of the environment and causing the accumulation of monocytes in subepithelial areas. The destructive effect of cathepsin D on bone was

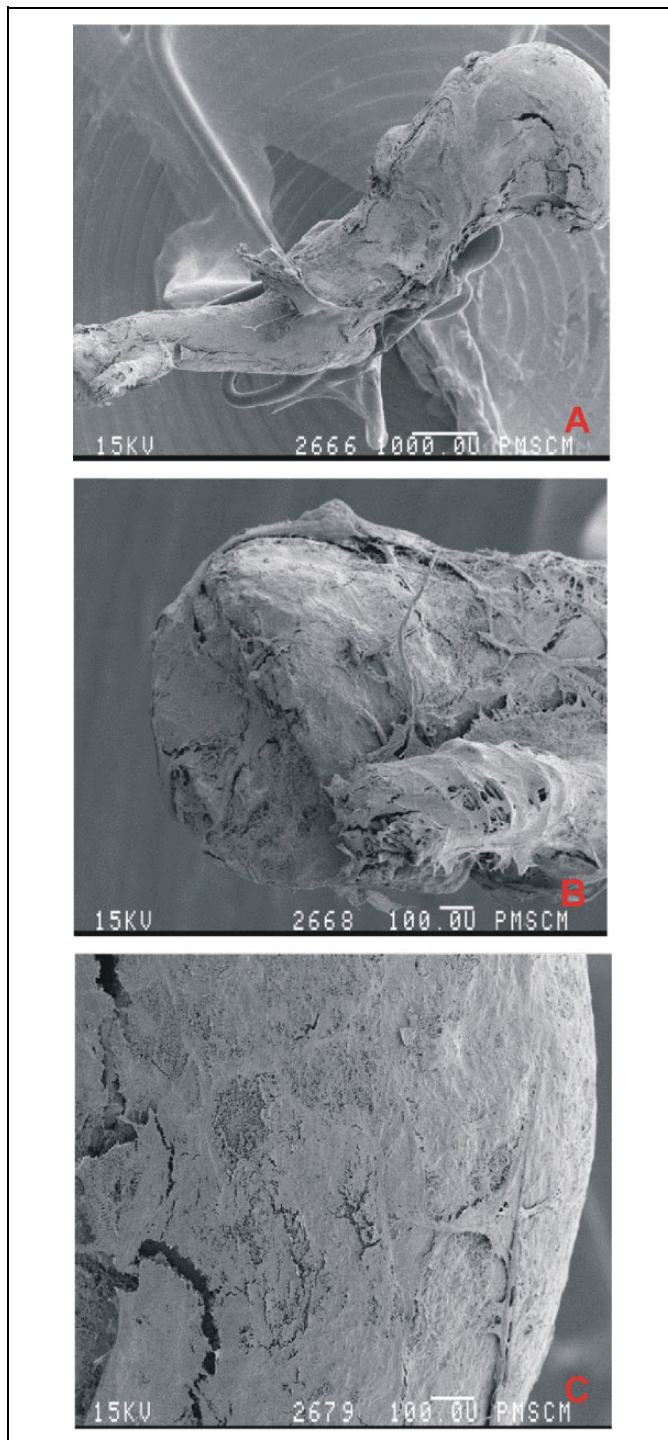


Figure 3. Chronic cholesteatoma otitis media. Removed malleus (group I, P2). Scanning electron microscopy showed a fragment of a damaged malleus (A) $\times 15$, distal part of the malleus handle (B) $\times 78$, head of the malleus (C) $\times 100$.

shown in 2002 by Chodynicky et al.¹¹ Based on this research, the most likely mechanism responsible for the process of bone resorption in cholesteatoma is the molecular cascade of osteoclast differentiation factors, which consist of OPG, the RANK (a protein belonging to the family of TNF receptors, regulated

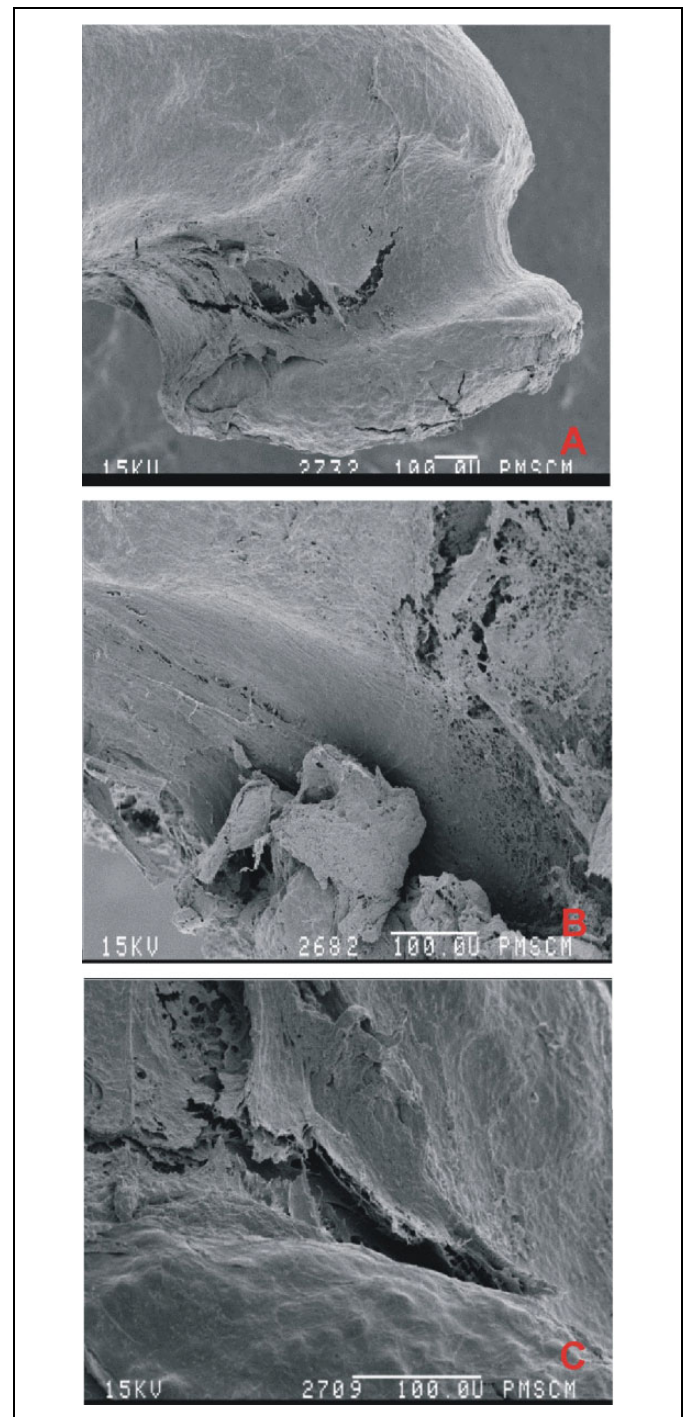


Figure 4. Chronic cholesteatoma otitis media. Removed incus (group I, P2). Scanning electron microscopy showed a long process $\times 60$ (A), a long process—articular surface $\times 200$ (B). Removed incus (group I, P5). Scanning electron microscopy showed eroded corpus of the incus $\times 300$ (C).

by IL-4 and transforming growth factor beta in T and cluster of differentiation 40 + lymphocytes), and the RANKL (a cytokine in the TNF family), forming the so-called OPG-RANKL-RANK triad. Osteoprotegerin, by binding with the RANKL protein, prevents binding with the RANK receptor and thus

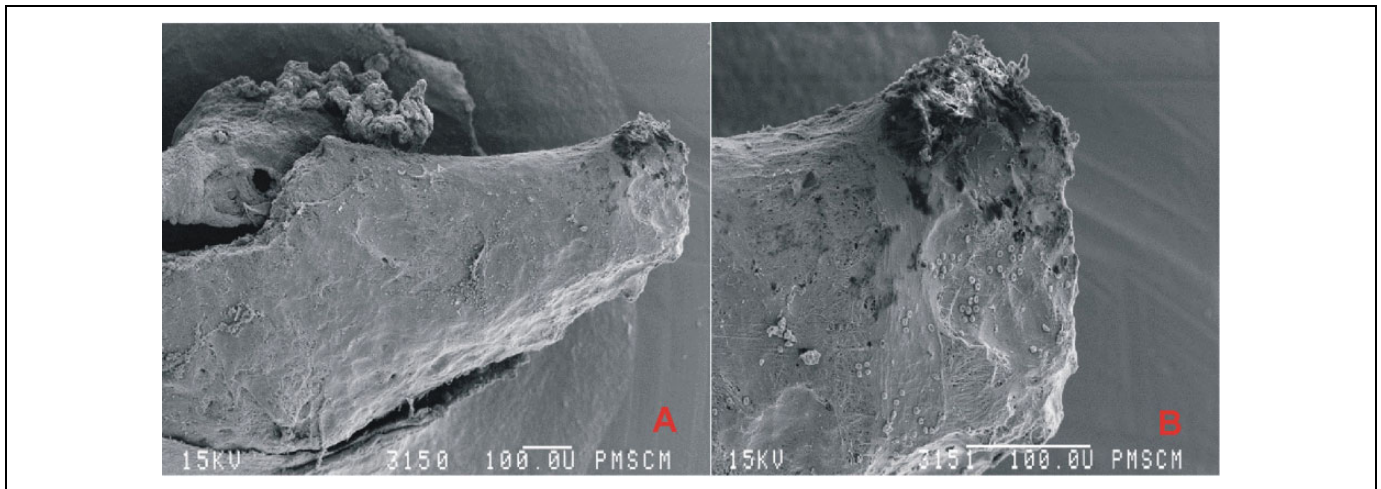


Figure 5. Chronic otitis media with granulation. A fragment of a damaged malleus (group II, P9). Scanning electron microscopy showed eroded handle of the malleus $\times 110$ (A), $\times 300$ (B).

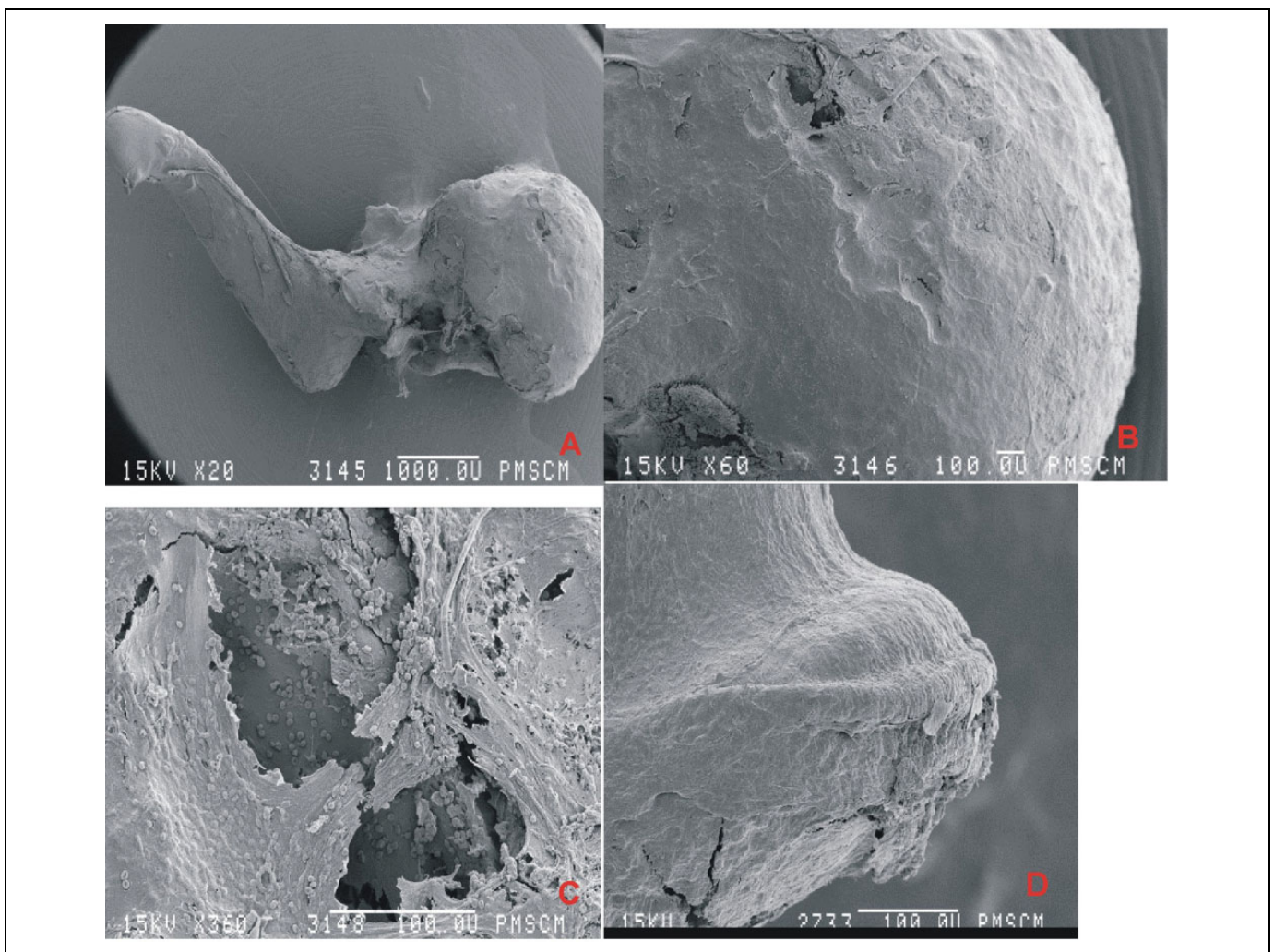


Figure 6. Chronic otitis media with granulation. Removed incus (group II, P11). Scanning electron microscopy showed damaged incus $\times 20$ (A), head of the incus $\times 60$ (B), eroded head of the incus $\times 360$ (C). Removed incus (group II, P14). Scanning electron microscopy showed eroded end of the long process $\times 160$ (D).

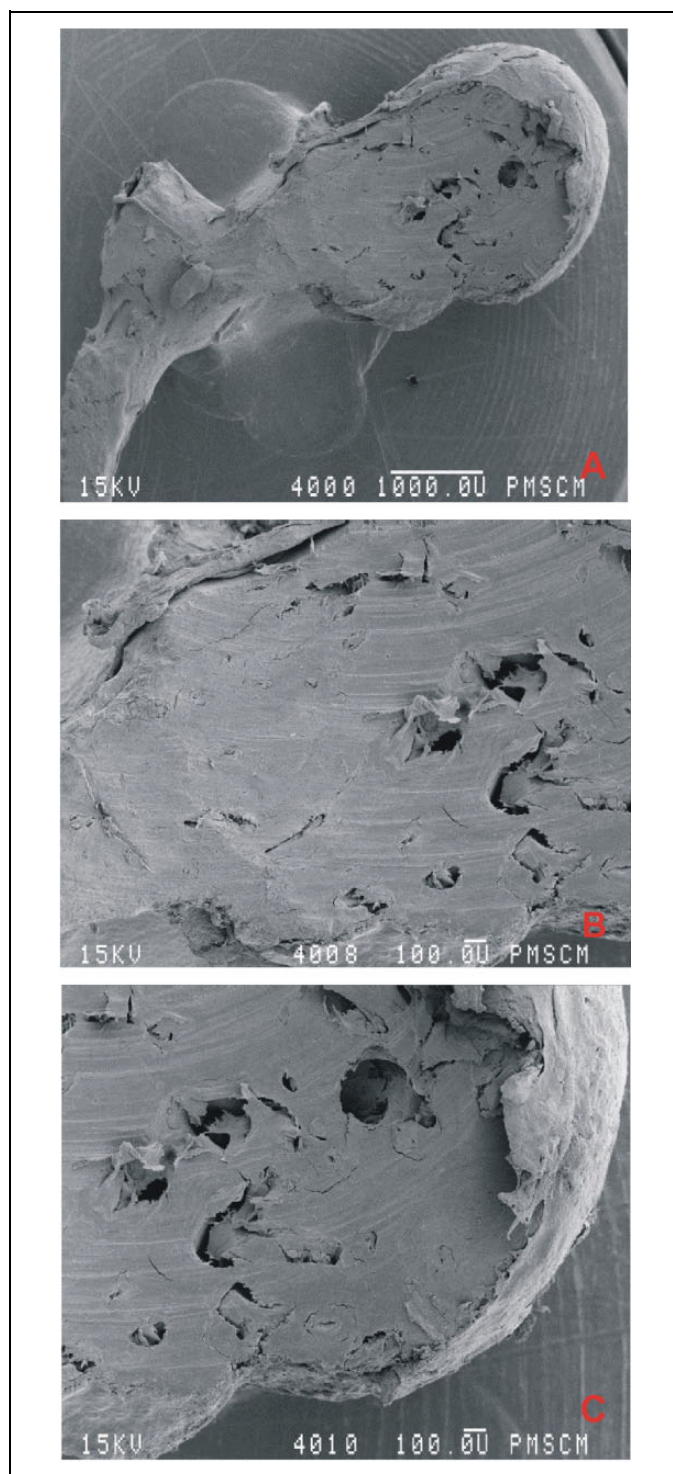


Figure 7. Chronic otitis media with granulation. Removed malleus (group II, P12). Scanning electron microscopy showed eroded malleus head $\times 20$ (A), $\times 48$ (B), $\times 48$ (C).

blocks the process of osteoclast maturation. The ratio of RANKL to OPG has been shown to be higher in cases of cholesteatoma than in a healthy ear. In 2000, Cinamon et al¹² presented an image of a bone adhering to the mass of cholesteatoma in the form of empty lagoons, ossification bands,

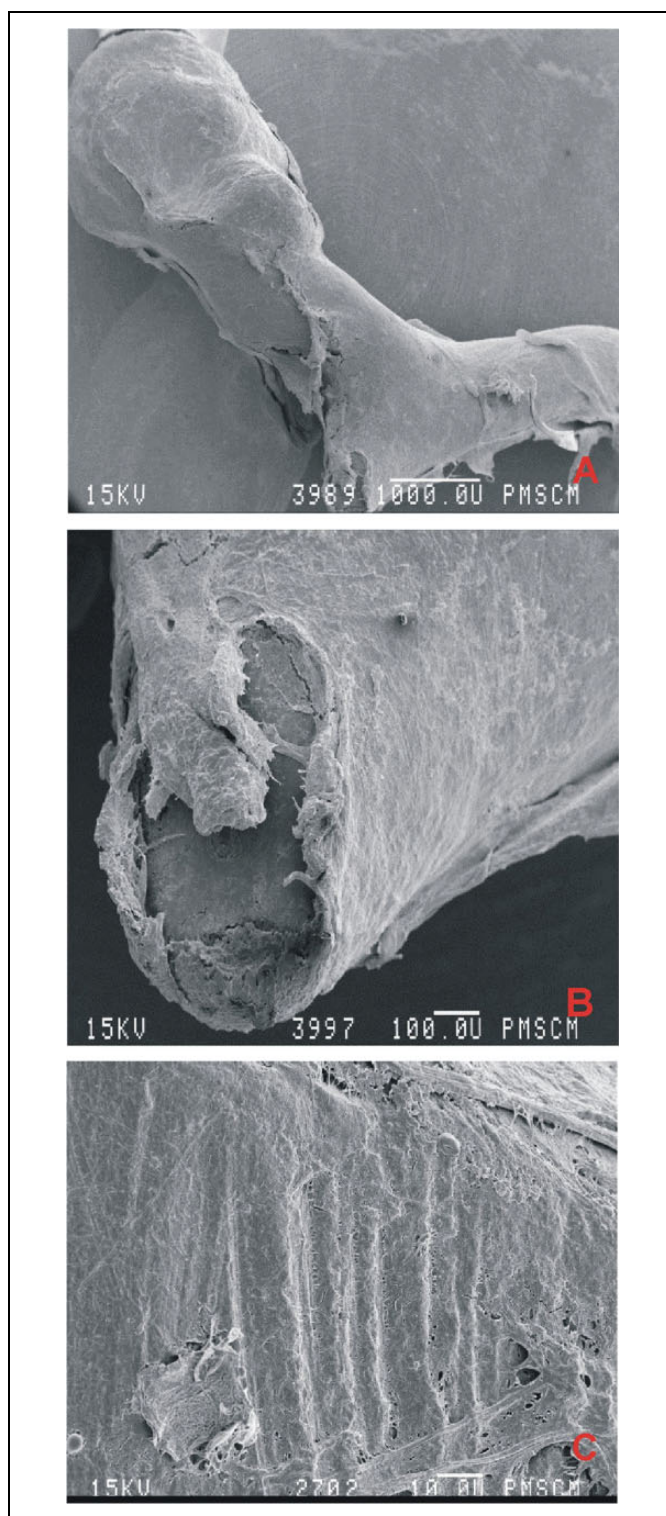


Figure 8. Simple chronic otitis media. Removed malleus (group III, P17). Scanning electron microscopy showed damaged malleus $\times 20$ (A), eroded lateral process $\times 100$ (B), eroded malleus surface $\times 200$ (C).

eosinophilic vesicles, and periosteal continuity disorders. In the mechanism of bone tissue damage in chronic otitis media with granulation, major roles are played by IL-1 α , TNF- α , and

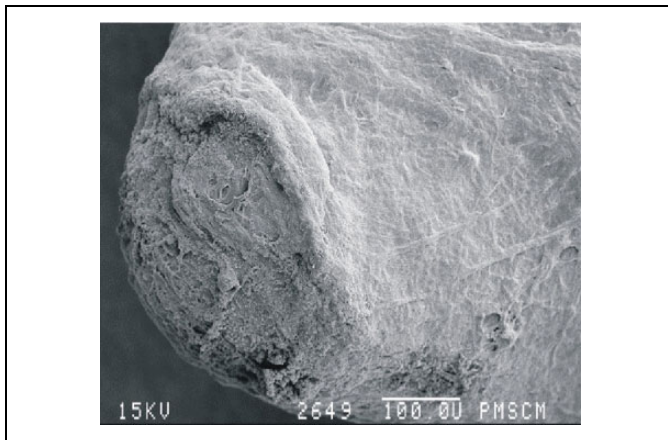


Figure 9. Simple chronic otitis media. Removed hammer (group III, P18). Scanning electron microscopy showed eroded short process $\times 200$.

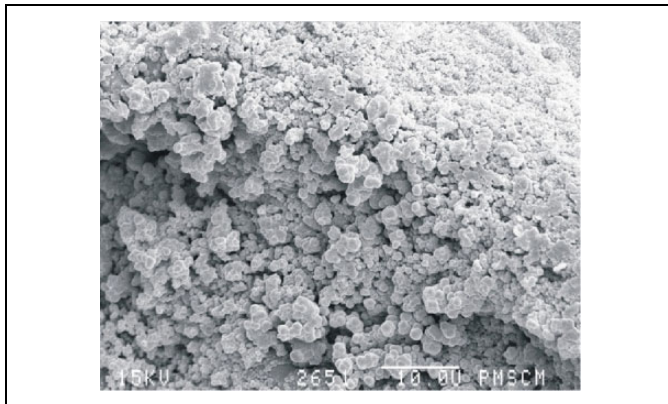


Figure 10. Simple chronic otitis media. Removed hammer (group III, P18). Scanning electron microscopy showed biofilm colonization $\times 2000$.

serine proteases, as well as the OPG-RANKL-RANK triad to a lesser extent, and the cascade of bone destruction, as in the case of chronic cholesteatoma of the middle ear, consists of several stages; however, it seems to be less complicated than that in the bone destruction process described above. Nevertheless, there

have been no detailed analyses, such as works on chronic otitis media with cholesteatoma. In all of the analyzed fragments of ossicles, we observed lesions and remodeling that indicate ongoing chronic inflammation. The largest changes were observed in cholesteatoma of the middle ear, where none of the analyzed ossicles were suitable for use in reconstruction of the middle ear conduction system. The lesions reached deep into the bone matrix and were observed at the highest magnifications ($\times 2000$ and $\times 4000$). We observed smaller lesions in chronic otitis media with granulation; however, the damage disqualified the possibility of using the material for reconstruction in these cases as well. The smallest lesions in the osseous tissue of the ossicles were observed in simple otitis media.

This study unequivocally shows superficial microdestruction of the ossicles observed by SEM even if the appearance under the surgical microscope was unchanged in patients with chronic cholesteatomatous otitis media. The above relationship was especially visible if the elements of the ossicular chain had close contact with a cholesteatoma lesion. Destruction to the surface of the ossicles prevents their use as a reconstructive material ossiculoplasty. Direct adhesion of the cholesteatoma to the ossicular chain seems to disturb the structure of the ossicles and change their features; thus, their use in tympanoplasty is controversial. Because of the different methods of preparing surgical specimens for SEM analysis, it is not possible to compare the presented results to those of previous studies by other authors.^{13,14}

Conclusions

Chronic inflammation involving the ossicles destroys individual elements of the auditory ossicular chain to various degrees, often preventing their use as a reconstructive material, despite their suitable macroscopic appearance.

The largest destructive changes in the ossicular chain were observed in chronic otitis media with cholesteatoma; smaller changes were observed in chronic otitis media with granulation. The process of bone destruction also occurs in simple chronic otitis media to a small degree.

In cases of severe damage to the ossicles inflicted by the inflammatory process, in ossiculoplasty, the otosurgeon should use material taken from outside the tympanic cavity (eg,

Table 2. Lesions and Damage to the Surface of the Examined Ossicles Observed by Scanning Electron Microscopy.

Type of Chronic Condition	Description of Damage Observed at Particular Magnifications			
	$\times 36$	$\times 100$	$\times 200$	$\times 2000$
Chronic suppurative otitis media with cholesteatoma	Crevised, deep bone loss	Deep, linear bone loss, irregular surface	Irregular surface with deep, linear bone loss; resorption of Howship's lacunae (?)	Extensive bone resorption
Chronic otitis media with granulation	Individual deep bone cavities, extensive resorption	Irregular bone loss, resorption of Howship's lacunae (?)	Irregular surface with shallow, linear bone loss	Irregular surface
Simple chronic otitis media	Irregular surface	Irregular surface	Small bone loss	Bacterial biofilm, smooth surface

? indicates resorption of Howship's lacuna.

modeled bone block made of a temporal bone) or an artificial prosthesis (eg, made of titanium or a biomaterial).


Declaration of Conflicting Interests


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